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ELECTROCONDUCTIVE POLYMER THIN FILMS WITH INTERNAL BIOACTIVE MOIETIES FOR BIOSENSOR APPLICATIONS

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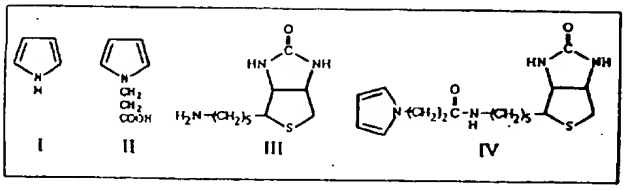
INTRODUCTION AND BACKGROUND

A major goal in the development of biosensors based on the transducer-active responses of electroconductive polymers is the conferment of biospecificity. Biospecificity implies the response of the transducer to a specific analyte through the actions of a biologically active indicator molecule such as an enzyme, antibody, DNA/RNA fragment, etc. We are interested in the development of conductimetric biosensors that exploit the very large and rapid change in electrical impedance that accompanies oxidoreduction of electroconductive polymers. Conductimetric biosensors have been well described[1]. Lawrence et al.[2] have used electrical conductivity changes to measure enzyme activity. The group of C. R. Lowe[3] described microfabricated conductimetric urea biosensors and Thompson et al.[4] have shown how this principle may be applied to immunoassays by tagging an antibody with urease. In these and similar cases, the bioactive indicator molecule generates ions that are detected by relative conductance changes ($\Delta G/G$). There has recently emerged considerable interest in the use of electroconductive polymers[5,6] as transducer-active materials in conductimetric chemical and biosensors. A general purpose conductimetric, H_2O_2-sensitive transducer has been developed that responds to H_2O_2-produced by oxidoreductase enzyme labels[7] and a novel analytical method for the interrogation of the conductimetric response of the electroconductive polymer thin film transducer has been described[8].

The transducer consists of an electropolymerized and covalently adhered[9] thin electroconductive multi-layered membrane on an interdigitated microsensor electrode array of defined cell constant[10]. In this paper we describe the addition of a third layer to the transducer that confers biospecificity via covalently immobilized biotin and imparts the capability to perform biotin-avidin/streptavidin binding bioassays. Two approaches were developed for conferment of biospecificity to the outer membrane layer. The first method involves copolymerization of pyrrole (I) with 3-(1-pyrrolyl)propionic acid (II) followed by direct conjugation of surface carboxylic acid groups with 5-(biotinamido)pentyl amine (III). The second method exploits direct linking of II with III to form a pyrrole-biotin conjugate (IV) followed by copolymerization of I and IV. The result in both cases is an internally bioactive polymer film.

Using biotin-oxidoreductase enzyme conjugates, a protocol may be developed, illustrated in Figure 1.a, in which the bound enzyme label produces H_2O_2 which then oxidizes the PPy film for conductimetric detection. Using the EPSIS method, these transducers were studied for their response to glucose.



EXPERIMENTAL

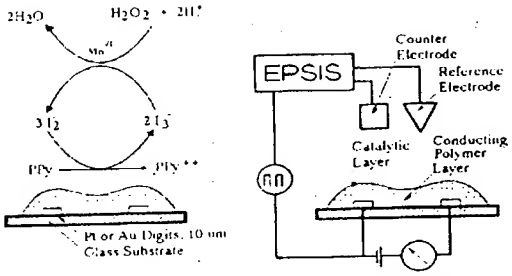
Transducer fabrication involved preparation of electropolymerized polypyrrole membrane films on microlitographically fabricated Interdigitated Microsensor Electrode (IME) arrays. The IMEs was fabricated from 1,000 Å of magnetron sputtered gold over 100 Å of adhesion promoting titanium/tungsten (Ti/W) on a chemically resistant, electronics quality, Schott D263 Borosilicate glass or oxidized silicon. The IME Au-1050-M-P (AAI-ABTECH) array possessed lines and spaces of 10 μm and consisted of 50 digit pairs each of 0.425 cm. The total interdigit area was 0.0425 cm^2 and the cell constant was 0.0471 cm^{-1}, on a chip that was ca. 1.0 cm^2.

Electroconductive polypyrrole-based membranes were grown by potentiostatic electropolymerization onto IME devices with interdigit spaces that were chemically modified with 3-aminopropyltrimethoxysilane and subsequently conjugated with 3-(1-pyrrolyl)propionic acid (3PyPA) using N-hydroxysulfosuccinimide (sulfo-NHS) (Pierce) and 1,3-diisopropylcarbodiimide (DIPC) (Chemical Dynamics). Electropolymerizations were done at +0.65 V *vs.* $Ag^0/AgCl$, 3 M Cl^- (RE803, AAI-ABTECH) applied to the shorted electrodes of the IME device using an EG&G PAR 173 Potentiostat and PAR 179 Digital Coulometer. The film was allowed to grow on each electrode and also between the digits of the pair of electrodes such that it formed an adherent and fully contiguous membrane[9]. The first electropolymerization bath contained 0.2 M pyrrole (Py), 2.5 mM poly(styrenesulfonic acid) (PSS), and 2.5 mM sodium dodecylbenzenesulfonate (DBS) at a pH = 3.0 and T = 20°C. The second catalytic layer was prepared from an electropolymerization bath that was neutralized by addition of 0.015% polyvinylamine (PVAm) and also contained 1 mg/ml poly(l-lysine) and 0.1 mM $Mo_7O_{24}^{6-}$ (Mo^{VI}).

Biospecific films were similarly prepared by electropolymerization of a third layer. In the first approach, the electropolymerization bath contained 0.1 M Py, 0.1 M 3PyPA, 2.5 mM PSS, and 2.5 mM DBS at a pH = 3.0 and T = 20°C. In the second approach, the third layer was prepared in an electropolymerization bath that contained 0.1 M Py, ca. 0.1 M 3-(1-pyrrolyl)propionic acid-5-(biotinamido)pentyl amine conjugate, 2.5 mM PSS, and 2.5 mM DBS at a pH = 3.0 and T = 20°C. The pyrrolyl-biotin conjugate (IV) was prepared by reacting 0.1 M 3PyPA with 0.1 M 5-(biotinamido)pentyl amine in the presence of 0.1 M sulfo-NHS and 0.1 M DIPC. At the end of each electropolymerization, the device was removed, rinsed thoroughly in 0.1 M KCl and characterized by cyclic voltammetry (CV), EPSIS™ conductivity testing, and its glucose sensor response determined using EPSIS.

Electrochemical characterization was done by cyclic voltammetry (CV) in deaerated 0.10 M KCl at 20°C using an EG&G PAR Model 273 Potentiostat. Single scans were performed at 10 mV/s over the range -1.0V to 0.00 V *vs.* $Ag^0/AgCl$, 3 M Cl^-.

Conductivity testing was performed in aqueous 0.1 M KCl using EPSIS™ (AAI-ABTECH). The test conditions were as follows: *Initialization Potential*: O.C. potential(V); *Initialization Period*: 0.0 min.; *Pulse Voltage*: 15 mV; *Pulse Duration*: 50 ms; *Pulse Delay Time*: 500 ms; *No. of Cycles*: 10 (ca. 5.5 sec). In these conductivity measurements, transducers were not electrolyzed away from their poise potential (hence 0.0 min. initialization period) and were interrogated for a total of 10 cycles or 5.5 sec. The measured conductance multiplied by the cell constant yields the electrical conductivity at the poise potential of the device.

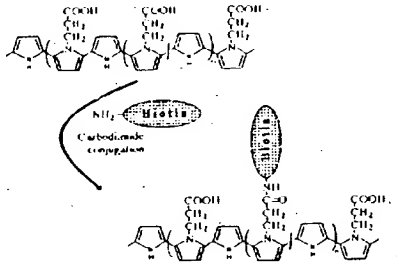


Scheme I

Figure 1. EPSIS sensor system.

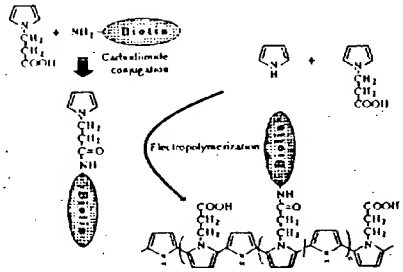
RESULTS AND DISCUSSION

Biospecific Films Previous work[3] has demonstrated the co-electropolymerizability of pyrrole and 3-(1-pyrrolyl) propionic acid. A 50 mole % solution of both monomers produced films that were electroactive and electrically conducting. While the final monomer ratio in the polymer relative to the polymerization solution is still unclear, indications are that the polymer is richer in pyrrole that in its N-acid derivative. Reaction of surface-available, N-acid moieties with 5-(biotinamido)pentyl amine using carbodiimide linking chemistry produces a biotinylated copolymer at the surface as shown in Scheme II.



Scheme II

Reaction of 3-(1-pyrrolyl)propionic acid with 5-(biotinamido)pentyl amine using carbodiimide linking chemistry produces a biotinylated pyrrole conjugate. Copolymerization of this pyrrole-biotin conjugate with pyrrole monomer produces a copolymer film that possess internal biotinyl moieties as shown in Scheme III.



Scheme III

Evidence for biotin availability at the surface of electropolymerized polypyrrole films was found by using a streptavidin-fluorescein conjugate and streptavidin-glucose oxidase conjugate. Streptavidin is an extracellular protein of *Streptomyces avidinii*, the functional portion of which has a molecular weight of ca. 47,000 daltons, consists of four identical subunits, and is tetravalent in its binding with biotin. Biotin is a common vitamin associated with the labile binding of carbon dioxide in various carboxylation cycles. Exposure of the biotinylated surface to the streptavidin conjugate results in strong binding of the protein to the vitamin as shown in Figure 2.

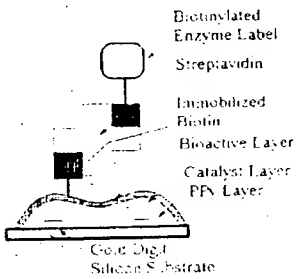


Figure 2. Schematic of the avidin-streptavidin binding reaction at the surface of an electroconductive polypyrrole-based biosensor.

A general purpose H_2O_2-sensitive, conductimetric transducer makes it possible to develop a wide range of oxidoreductase enzyme biosensors such as those based on glucose oxidase.

$$\beta\text{-D-Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose oxidase}} \text{D-Gluconic acid} + H_2O_2$$

A PPy-based, conductimetric bio-transducer that is sensitive to H_2O_2 can readily configured into an immunosensor by conferring the transducer with the specificity of biotin and exploiting strong biotin-streptavidin binding in various bioassays. The EPSIS method provides a convenient approach and the EPSIS instrument a convenient platform for the development of biospecific oxidoreductase enzyme biosensors and for the fabrication of oxidoreductase labeled immunosensors.

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